CHROMOSOMES AND THE LIFE CYCLE OF HYDATINA SENTA.[1]

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A reëxamination of the chromosomes of this rotifer seemed desirable owing to the results of some of my early experiments (Shull, '12), in which it was shown that whether one of these rotifers is to be a male-producer or female-producer is irrevocably decided within a period several hours in duration occurring in the course of maturation of the parthenogenetic egg from which the individual develops. Influences acting upon the egg before and after that period were found to be without effect. This result indicated that some very brief event occurring in maturation decided the fate of the female arising from the egg, and it seemed possible that the event might be a change in the number of chromosomes, perhaps comparable to the chromosome change in the aphids and phylloxerans, in which the diminished number in the male is still greater than the haploid. Sharp distinction between male-producers and female-producers is known in the phylloxerans without any difference in the chromosome number (Morgan, '09); but in these insects the time at which the differentiating event occurs is unknown.

A difference in the number of chromosomes between male-producers and female-producers was not excluded by the results of previous studies of the cytology of *Hydatina*. Lenssen ('98) states the number of chromosomes to be 10 or 12 in the female-producing egg. In one passage it is plain that these numbers imply uncertainty as to the correct number. Perhaps uncertainty was intended to be implied by the other passages in which the expression "10 or 12" is used, but there is room in one case to suppose that Lenssen observed sometimes 10, sometimes 12,

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in different eggs. If such differences existed, they might represent the two types of female. The possibility that Lenssen believed the number of chromosomes to be different in different individuals is heightened by his statement that the reduced number in the egg of the male-producer is "probably five." This would suggest 10 as the diploid number in the male-producer, though maturation phenomena might reduce the haploid number to less than half the diploid.

Whitney's ('09) studies threw no light on Lenssen's statements, for he was unable to count the chromosomes with accuracy. Using American specimens, he arrived at a diploid number between 20 and 30, the highest number actually counted being 25. There was nothing in the male egg or fertilized egg to render the count more definite, for the number discovered in the male egg ranged from 10 to 14, and in the fertilized egg, during maturation, 14 was the largest number seen.

In view of the uncertainty of these determinations, and of the possibility that the male-producing and female-producing females might differ in their chromosome number, I undertook a re-examination of this difficult material. Notwithstanding the difficulties, I have a fair degree of confidence in the conclusion reached.

Preparation of Material.

Although several fixing agents were used, the specimens that were good enough to study were all fixed in Bouin's fluid. Dehydration was accomplished by the drop method. When the objects had reached 70 per cent. alcohol, they were carefully wrapped in bits of stratum corneum of frog skin, which had shortly before been put into 70 per cent. alcohol, in order to make the objects large enough to imbed easily. The frog skin was held in compact form by fragments of cover glass until the objects were in 96 per cent. alcohol, by which time the skin had hardened so that it would not unroll. Six to eight rotifers were as a rule wrapped in each roll. Sections were all cut 5 μ in thickness. All were stained in iron-hematoxylin. They were also lightly stained with eosin, but not for the purpose of this study. Unfortunately the chromosomes show a strong tendency

to collect in masses, so that the number of specimens clear enough to furnish counts was not large.

General Course of Maturation.

Inasmuch as I was primarily interested in the number of chromosomes, in each of the kinds of females, I have made no attempt to render a complete account of maturation. Such facts as have been revealed, even though incidental to the main object, are here recorded. Only that part of the maturation which occurs in the oviduct of the female has been studied. The completion of the process after the egg is laid has not been followed.

About the time when the oöcyte has reached its maximum size and is fairly fixed in form (ellipsoidal), not yielding to the movements of the body of the female, the nucleus rapidly increases in volume. An aster appears in the cytoplasm near it, being readily visible by the practical absence of yolk spherules, although radiations are very indistinct. So plain is the aster that it was invariably used as a guide-post in locating maturation spindles. The spindle is formed within the nucleus. Upon it the chromosomes appear as long slender threads tapering toward both ends (Fig. 1). As the spindle develops the nuclear membrane disappears, so that the spindle is out in the cytoplasm: but there is always a definite space in the cytoplasm in which the spindle is located so that there is no confusion of chromosomes and yolk spherules. With very few exceptions, the spindle, which is near the periphery of the cell, is turned toward the intestine. Exceptions to this rule are more common in male-producers than in female-producers.

In the female-producing egg the chromosomes arrange themselves on the equator. In this condition the cell appears to remain until the egg is laid, for out of a large number of specimens not one was found that had proceeded beyond that stage. Lenssen ('98), indeed, concluded that the division was never completed; but Whitney ('09) observed the single polar body at the periphery of the segmenting egg.

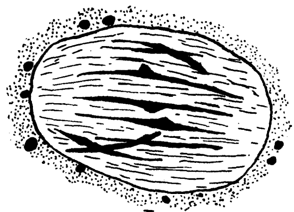
In the male-producing egg several specimens indicate that the chromosomes meet in pairs on the equator of the spindle. The

pairs separate into their components, each group proceeding to the end of the spindle. In this late anaphase the cell appears to remain until the egg is laid, for out of many specimens none was found in later stages.

Number of Chromosomes.

The tendency of the chromosomes to adhere to one another in masses has rendered the determination of their number difficult. Fortunately in my material a number of specimens appear to agree in the number, and this is the largest number found. In such specimens the chromosomes are of approximately uniform size, while in cells showing smaller numbers some chromatin masses are nearly always distinctly larger than others, indicating that the larger bodies are probably compound. From a study of this material I conclude that the diploid number of chromosomes is 12. In female-producing parthenogenetic eggs, the best stage for counting the chromosomes is just before or during the formation of the equatorial plate, because the chromosomes are then well-defined, and are not so crowded as later in the equatorial plate. Such a stage is represented in Fig. 2.

The chromosomes of the male-producing egg can be counted fairly well at several stages. As in the female-producing egg, one favorable stage is just before the formation of the equatorial plate. In the equatorial plate the chromosomes can also be counted, in these eggs, because the chromosomes unite in pairs, hence there is a smaller number of discrete objects, and they are not so crowded. Figure 3 shows distinctly six pairs. In this figure the equatorial plate is viewed obliquely, and the pairs are not at the same level, a fact not shown in the drawing. In Fig. 4 there are six bodies, of which three show signs of bivalence. The others may be bivalent, but appear single because viewed from the end of the spindle. In late anaphases of the male-producing egg the chromosomes are always crowded. Five chromosomes can usually be recognized, but some specimens show six. From the existence of six pairs on the equatorial plate, I conclude that the number must be six even where only five can be recognized. The late anaphases which are favorable



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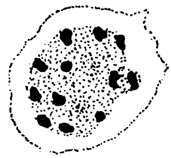

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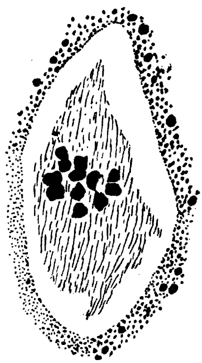

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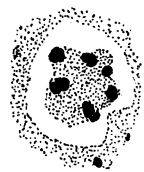

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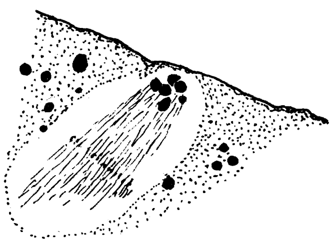

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FIG. 1. Maturation spindle of male-producing egg being formed within nucleus. A distinct nuclear membrane is still present. Only part of the spindle is in this section.

FIG. 2. Equatorial plate of maturation spindle of female-producing egg.

FIG. 3. Metaphase of first maturation spindle of egg of a male-producer. The spindle is viewed obliquely from the side, and the chromosomes are not all at the same depth in the specimen.

FIG. 4. Metaphase of the first maturation spindle in a male-producing egg. The view is nearly polar. Three of the chromatic bodies, set obliquely, give indications of being bivalent; the rest appear single.

FIG. 5. Portion of first maturation spindle, in late anaphase, of male-producing egg. The spindle is cut obliquely, and the outer end is here shown.

FIG. 6. Inner group of chromosomes, six in number, in late anaphase of first maturation division of male-producing egg.

enough to count include one or more groups at both outer (Fig. 5) and inner (Fig. 6) ends of the spindle, and the number is six in either case.

Discussion.

From the best evidence I have been able to obtain, the number of chromosomes in *Hydatina senta* must be placed at 12. The number appears to be the same in male-producers and female-producers. This conclusion is less illuminating in some respects than might have been expected, but simplifies the probable chromosome behavior in certain parts of the cycle. The very sharply defined distinction between male-producers and female-producers, and the irrevocable determination of the nature of the egg at the time of maturation, would be regarded as "explained" if it could be shown that they rested on chromosome differences. If there are such differences, they are not differences of number. Whatever change occurs at maturation, to distinguish eggs that yield male-producers from those that yield female-producers, must, however, be quite as definite an event as the loss of a chromosome to the polar body.

With regard to the male phase of the life cycle, one may surmise the following consequences of the uniformity of chromosome number. The male probably develops with the haploid number of chromosomes, and in the maturation of his spermatozoa the reduction division is either abortive or suppressed. Or the male may at some stage double the number of chromosomes and maturation include a numerical reduction. In either case, the spermatozoa should contain six chromosomes. The sexual egg (identical with the male-producing egg described in this paper) presumably also has six chromosomes. At fertilization the number is restored to 12.

The status of the females hatching from fertilized eggs, which may be called the stem mothers, is a peculiar one. These stem mothers have been shown by hundreds of determinations to be always female-producers (Shull, '12). Whether, in the absence of differences in chromosome number, the feature which distinguishes a female-producer derived from a parthenogenetic egg from a male-producer is also the feature which makes a stem mother a female-producer can only be conjectured at present.

I am not prepared to advocate any view as to the method by which, in the absence of visible chromosome differences, a female-producer is distinguished from a male-producer. It has been suggested to me that there may be a difference in the chromatin, perhaps a quantitative difference, which does not involve the number of chromosomes. This possibility may be investigated in other ways. If there is a single fundamental distinction between the two kinds of females, any attempt to discover it must take account of the fact that the distinguishing feature of the female-producing parthenogenetic egg must also be an attribute of the fertilized egg.

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